

Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life

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Sexual reproduction and clonality in eukaryotes are mostly seen as exclusive, the latter being rather exceptional. This view might be biased by focusing almost exclusively on metazoans. We analyze and discuss reproduction in the context of extant eukaryotic diversity, paying special attention to protists. We present results of phylogenetically extended searches for homologs of two proteins functioning in cell and nuclear fusion, respectively (HAP2 and GEX1), providing indirect evidence for these processes in several eukaryotic lineages where sex has not been observed yet. We argue that (i) the debate on the relative significance of sex and clonality in eukaryotes is confounded by not appropriately distinguishing multicellular and unicellular organisms; (ii) eukaryotic sex is extremely widespread and already present in the last eukaryotic common ancestor; and (iii) the general mode of existence of eukaryotes is best described by clonally propagating cell lines with episodic sex triggered by external or internal clues. However, important questions concern the relative longevity of true clonal species (i.e., species not able to return to sexual procreation anymore). Long-lived clonal species seem strikingly rare. We analyze their properties in the light of meiotic sex development from existing prokaryotic repair mechanisms. Based on these considerations, we speculate that eukaryotic sex likely developed as a cellular survival strategy, possibly in the context of internal reactive oxygen species stress generated by a (proto) mitochondrion. Thus, in the context of the symbiogenic model of eukaryotic origin, sex might directly result from the very evolutionary mode by which eukaryotic cells arose.

reactive oxygen species | evolution | protists | eukaryotes | sex

The prevailing view seems to be that multicellular organisms are obligately sexual while clonally reproducing representatives are an oddity, whereas microbial eukaryotes (protists) are perceived as just facultatively sexual, or even purely clonal. However, a closer look suggests this to be a rather artificial distinction stemming from improper comparison of unicellular and multicellular individuals, which are different entities. In fact, a multicellular organism is “nothing else than” a clonally propagating population of cells that are physically linked. Hence, from the perspective of cell lineage, sex in multicellular organisms is as episodic as it is in facultatively sexual unicellular eukaryotes. Indeed, tens to hundreds of clonal cell divisions may occur in the germ line of different multicellular organisms (1). Of course, the frequency with which a clonally reproducing cell lineage embarks on sex (i.e., cell fusion-making diploid or meiosis-producing haploid cells), and the clues triggering sexual behavior, vary greatly among organisms. These differences, however interesting (and making organismal biology such a wonderful pursuit), are secondary to our main point, which is that sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. Below we discuss evidence, partly derived from our own observations, documenting the ubiquitous, ancestral presence of sex in eukaryotes. We do not consider this a coincidence, as sex might be inherently linked to the way in which eukaryotic cells as such came into being.

Sex in Eukaryotic Microorganisms: More Voyeurs Needed

Whereas absence of sex is considered as something scandalous for a zoologist, scientists studying protists, which represent the majority of extant eukaryotic diversity (2), are much more ready to accept that a particular eukaryotic group has not shown any evidence of sexual processes. Although sex is very well documented in many protist groups, and members of some taxa, such as ciliates (Alveolata), diatoms (Stramenopiles), or green algae (Chloroplastida), even serve as models to study various aspects of sexuality (3–5), direct observation of sexual processes is lacking for a majority of protist species (6, 7), and entire protist lineages cannot be considered sexual with any certainty yet. However, information about the life history of many protists is rudimentary, and a strong bias, especially toward parasitic lineages, is also found in the selection of current genome sequencing projects (8). We screened the literature to find evidence in individual principal phylogenetic lineages of eukaryotes for “signs of sex” (9), including physical observation of cell and/or nuclear fusion, cytological or genetic evidence for meiosis and/or recombination, or changes in ploidy levels during the life cycle. The following lineages, admittedly often poorly studied with regard to their biology in general, might still be truly asexual in as far as we know today (Figs. 1 and 2): Ichthyosporidia, Cristidiscoidea, Apusomonadida, Breviatea, Ancyromonadida, Mantamonadida, Rigifilida, Collodictyonida, Telonemia, Centrohelida, Palpitomonadida, Katablepharida, and Picozoa (for the phylogeny of many of these groups, consult Box 1; representatives of some of these lineages are displayed in Fig. 1). However, Jakobida, Glaucophyta, and Malawimonadida, thus far unreported to exhibit sex, all contain genes involved in plasmogamy (gamete fusion) and/or karyogamy (nuclear fusion; Fig. 2).

It has been argued that the putative asexuality of many eukaryotic microbial taxa might be an “observational artifact” (6), as signs of sex are often very difficult to discern. Numerous examples illustrate how little we know about the actual life of eukaryotic microorganisms, even those studied for a very long time. The kinetoplastid protozoan *Trypanosoma brucei* (Euglenozoa), a causative agent of sleeping sickness, is one of the best-studied protist species, yet meiosis and gametes were only detected very recently (10, 11). Although meiosis has thus far been considered to be very rare in trypanosomes, it may actually

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be more frequent, as both intraclonal and interclonal mating has been documented (10). The sexual cycle of the ascomycete fungus *Aspergillus fumigatus* was described only in 2009 (12), i.e., nearly 150 y after the species was originally described, despite the fact that this ubiquitous causative agent of life-threatening invasive aspergillosis and important allergen causing severe asthma and sinusitis had been extensively studied for years (13). Many factors make detection of sex challenging in most groups of microbial eukaryotes. These include the small size of most of the organisms, the fact that many cannot be cultivated, the absence of appropriate environmental stimuli under laboratory conditions, the lack of suitable mating partners, or the low frequency of sex manifestation (6, 7). It is possible that signs of sex are often displayed, yet we do not realize what they actually signify. The taxonomic history of algae and fungi is full of examples of organisms, originally considered as completely different taxa, turning out to be different sexual life cycle stages. Fungi frequently exhibit so-called pleomorphism, i.e., occurrence of morphologically distinct asexual and sexual phases, anamorphs and teleomorphs, respectively, of the same species. A huge number of anamorphs and teleomorphs have been described as separate taxa, but are now being unified using DNA sequence data (14). Among algae, one of the best-documented cases is that of prymnesiophytes, which were found to exhibit a biphasic haplo-diploid life cycle with morphologically differentiated stages (e.g., bearing scales of a different type) that had previously often been classified as different species or even genera (15, 16). Dimorphic sexual life cycles may indeed be much more common than is currently acknowledged. Myxozoans (Metazoa) are a good example. Mostly parasites of fish, myxozoans were for almost a century considered to be protists until molecular data revealed that they are probably extremely reduced cnidarians (17). This analysis was further borne out by shared antigenicity of their polar filaments with other cnidarians (18). During their complex life cycle, they also use invertebrate hosts, usually oligochaetes, in which they develop into a morphologically distinct actinosporean. The latter formerly constituted a class of its own, now void (19). Recent studies showed that meiosis, followed by a highly complex development of a consortium of haplo-diploid cells and delayed fertilization, indeed occurs in the actinosporean part of the life cycle within the oligochaete host (20). Good candidates for dimorphic sexual life cycles are found in cryptophytes (Cryptomonadida), an algal group remarkable for their structurally complex plastids representing an integrated red algal endosymbiont (21). Reports on signs of sex in cryptophytes have been very limited. A detailed account on gamete mating, including cell and nuclear fusion, was published only for a single species (*Chroomonas acuta*) (22), whereas meiosis has still not been reported. In another species (*Proteomonas sulcata*), two alternating morphologically different forms were observed, the larger one with twice as much DNA as the smaller one and hence most likely representing the diploid stage (23). Interestingly, evidence, or at least hints, for the occurrence of similar morphological dimorphism (alas without data on the ploidy level) exists for several other cryptophyte lineages, which led to the speculation that cryptophytes may exhibit an ancestral cryptic sexual dimorphic life cycle (24, 25), their name thus possibly being a nice instance of “nomen est omen.” A further remarkable case in point concerns two other curious algal groups in Stramenopiles. The first is the order Parmales, an enigmatic group of small planktonic spherical microalgae covered by siliceous plates known since the 1980s, but until recently without cultured representatives and with effectively unknown phylogenetic position (26). The second is the class Bolidophyceae comprised of minute naked marine flagellates discovered 15 y ago and shown to be a sister group of diatoms (27). In 2011, both groups were tied together by the amazing finding that the first (and thus far only) molecularly characterized parmalean species is phylogenetically nested among bolidophytes (28). This led to the hypothesis that the different bolidophyte and parmalean morphotypes are actually alternating stages of a biphasic life cycle of the

same algal group (26, 28). In analogy (or as a matter of fact perhaps homology) to diatoms, the flagellated bolidophytes represent the haploid stage (which was recently supported by a transcriptomic study of a bolidophyte isolate) (29), whereas the armored coccoid Parmales probably represent the diploid stage. However, both the presumed mating of the bolidophyte flagellates and meiotic division of the parmalean coccoids have not been observed yet.

We discussed these examples at length because they remind us of the fact that we still have a tendency to underestimate how widespread sexual practices are in the different eukaryotic groups. With the advent of high-throughput genome sequencing projects, much more information regarding the abundance of sexual procreation will come to light. However, much more attention should also be paid to studying life histories of protists to achieve a more satisfactory level of understanding of the actual everyday life in this fascinating realm.

Genome Sequencing Supports the Fundamentally Sexual Nature of Eukaryotes

As indicated, a major source of signs of sex for any species is its genome. Thus, the recent rapid growth of genomic data from diverse protist lineages holds a lot of promise for reconsidering their status as asexual. Several types of signs of sex to be gleaned from genome sequences have been discussed (30). Among them, the “meiosis detection toolkit” is the most readily applied (9). This consists of checking whether gene orthologs functionally associated with (or even specific for) meiosis are present. Indeed, this approach has become highly efficient in uncovering the secrets of the intimate life of eukaryotes. Let us consider several illuminating examples.

Choanoflagellates are an extremely interesting protist group, as they are the closest living relatives of multicellular animals (Metazoa) (31). They comprise some 250 described species of solitary or colonial bacterivores capturing prey with the aid of a collar of microvilli around a single posterior flagellum. Although studied for more than 150 y, sex had remained undiscovered for this group. A new chapter in choanoflagellate biology started with sequencing the genome of *Monosiga brevicollis* (32). The first hint for the occurrence of (occasional) sex in *M. brevicollis* came with the detection of active long terminal repeat (LTR) retrotransposons (33), because it has been hypothesized that asexual organisms lack mechanisms sufficient to keep retrotransposon proliferation under control, explaining why retrotransposons are missing from ancient (i.e., evolutionarily stable) asexuals (34). An even more persuasive line of evidence came in the form of the identification of genes of the meiosis detection toolkit in *M. brevicollis*, suggesting the choanoflagellate to be capable of meiosis (35). Clinching evidence for the sexual nature of choanoflagellates was next reported by Levin and King (36), who showed a distantly related species, *Salpingoeca rosetta*, exhibiting a sexual life cycle with switching between haploid and diploid stages mediated by anisogamous mating, even catching the event on film. Haploid cultures of *S. rosetta* became diploid (i.e., had sex) in response to stress, in this instance taking the form of nutrient limitation. Another example is offered by the marine planktonic coccoid green alga *Ostreococcus tauri*, considered the smallest free-living eukaryote described to date (37). Sexual processes (gamete mating or meiosis) have never been observed in *O. tauri* or its relatives, although meiotic genes were found in its genome (37). A subsequent population genetic study, using eight nuclear markers on two chromosomes, gave obvious indications for genetic recombination in *O. tauri* (38). Interestingly, these authors find sexual encounters to be infrequent and estimate the relative rate of meioses/mitoses among their strains to be 10^{-6} . Population-wide analyses of organellar genomes further revealed recombination of plastid genomes approximately one-third as frequent as recombination of nuclear genomes, suggesting a relatively frequent biparental inheritance of the plastid (but not mitochondrion) in *O. tauri* (39). Also the double nuclei containing diplomonads such as *Giardia intestinalis* (Fornicata) have mostly been assumed to be asexual. However, on closer genomic inspection, allelic differences have been found,

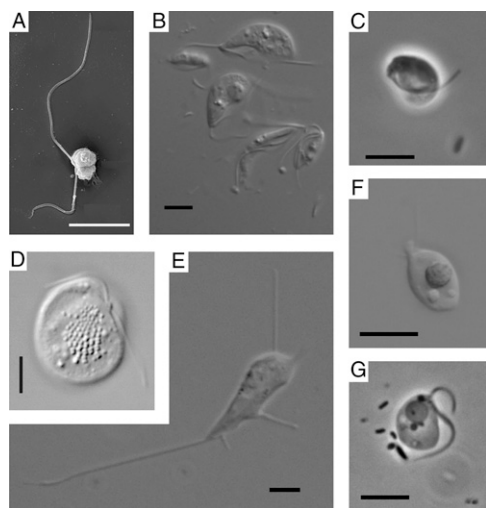


Fig. 1. Representatives of deep eukaryotic lineages without published evidence for sex thus far. (A) *Picomonas judraskeda* (Picozoa). (B) *Andalusia incarcerata* and another, thus far undescribed jakobid (Jakobida). (C) *Ancyromonas sigmoidea* (Ancyromonadida). (D) *Rombia truncata* (Katablepharida). (E) *Breviata anathema* (Breviatea). (F) *Telonema subtilis* (Telonemia). (G) An undescribed malawimonad (Malawimonadida). Images courtesy of Petr Táborský (B), Aaron Heiss (C, E, and G), and Akinori Yabuki (F); A and D were adapted from refs. 118 and 120. (Scale bars, 5 μ m.)

indicative of recent, although again rare, sex (40). Different chromosomal loci from several *G. intestinalis* strains gave alternative phylogenies, showing that they do not share evolutionary history and individual loci analysis indeed supported meiotic recombination (41). The use of the meiosis detection toolkit in *G. intestinalis* implies it being sexual as well (42).

The list of eukaryotic species that lack strong direct evidence for meiotic sex, but that seem sexual, as suggested by the presence of these meiosis-associated genes, is growing longer and longer. It includes the parabasalid *Trichomonas vaginalis* (43), the heterolobosean *Naegleria gruberi* (44), various trebouxiophyte green algae (45), Microsporidia (Opisthosporidia) (46), Glomeromycota (arbuscular mycorrhizal fungi) (47, 48), and the naked foraminiferan *Reticulomyxa filosa* (Rhizaria) (49). It also comprises the amoeba *Capsaspora owczarzaki*, representing Filasterea, a small lineage related to multicellular animals and choanoflagellates, which have far more diverse proteins for cell adhesion and transcriptional regulation than encoded by the choanoflagellate genome (50). These gene sets probably allowed the transition from unicellular organisms to metazoans. Meiosis-associated genes are also found in *Symbiodinium* (Alveolata), this dinoflagellate probably having sex in its rarely observed free-living state while being asexual in marine symbiosis (51). Finally, well-studied parasitic protists from highly diverse lineages of amoebozoans, apicomplexans (Alveolata), and kinetoplastids are also giving up their sexual secrets. Many of them show sex to be induced under the stress condition of having to leave their host (52).

Further Genome Sequencing Support for Sex: Cell and/or Nuclear Fusion Factors

In the excitement over the recognition of meiosis-associated genes all over the eukaryotic phylogenetic tree, one might forget that fusion of both cells and nuclei is also critical during the sexual cycle. However, gamete fusion (plasmogamy) and nuclear fusion (karyogamy) have been studied much less than meiosis. Although there is quite some variation in fusion factors, HAP2 (GCS1) protein homologs are implicated in plasmogamy in distantly related eukaryotes. Highly diverse eukaryotic gametes carrying loss-of-function mutations in HAP2 fail to fuse. This protein family could thus indeed represent an ancestral gamete fusogen in eukaryotes (53, 54). Where nuclear fusion

is concerned, the GEX1 protein family (fungal orthologs: KAR5) has recently been found to represent a potentially conserved karyogamy factor (55). We decided to investigate the phylogenetic distribution of HAP2 and GEX1 homologs in newly available genomic datasets, including data from our ongoing genome projects for two especially interesting eukaryotic lineages: jakobids and malawimonads (see *SI Text* for details). Both HAP2 and GEX1 can apparently be traced back to the last eukaryotic common ancestor (LECA), and their presence in malawimonads and jakobids provides the first indication for sex in these lineages (Fig. 2). The identification of GEX1 homologs in glaucophytes (*Cyanophora paradoxa*) may also be the first sign of sex reported for this group. One important caveat has to be mentioned, however: retention of plasmogamy and karyogamy factors in itself does not necessarily imply the presence of meiotic sex, as parasexual processes such as those observed in some fungi also rely on these factors. However, this would still count as sex because (more limited) recombination occurs. On the other hand, the absence of discernible HAP2 homologs from genomes of several protist lineages (Fig. 2) should not be interpreted as unambiguous support for their possible asexual nature, because

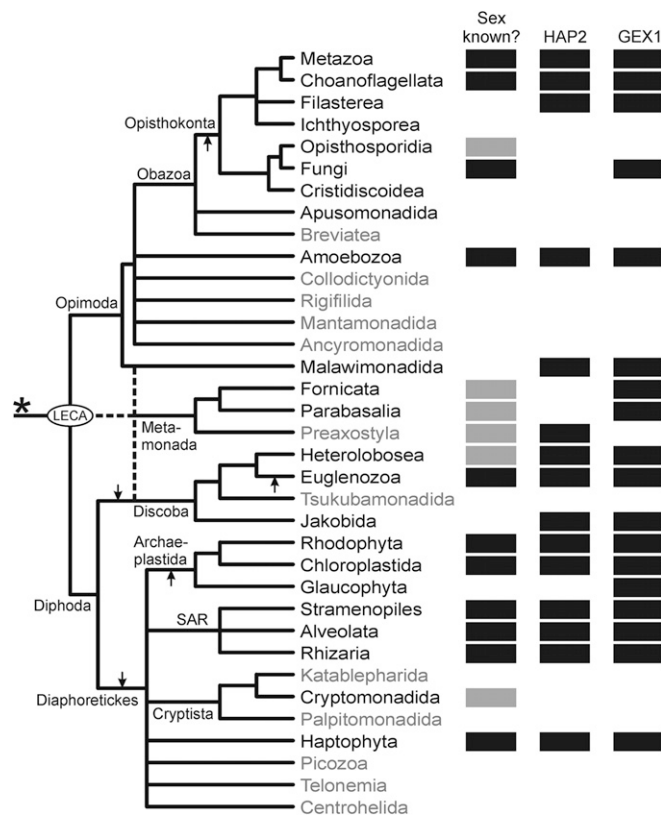


Fig. 2. Distribution of (meiotic) sex and selected sex-related features in eukaryotes. The schematic phylogeny is a consensus of recent literature (Box 1). Root position according to Derelle et al. (138); root positions suggested by others (132–136) indicated by small arrows. The Metamonada position remains unsettled. *Uptake of an α -proteobacterium at the origin of the eukaryotes. For each lineage, previously reported or assumed presence of sex is indicated: black boxes, well-documented sex; gray boxes, limited evidence for sex (rare direct observations, indirect inference from genomic data); no boxes, no published evidence; references used here are given in *SI Text*. The distribution of homologs of HAP2 and GEX1 proteins (implicated in gamete fusion and karyogamy, respectively; see main text) was obtained searching public and private genome and transcriptome resources (see *SI Text* for further details of genes identified). Absence of boxes does not directly imply absence in a lineage, especially for those given in gray, with limited (or absent) genome-scale sequence data.

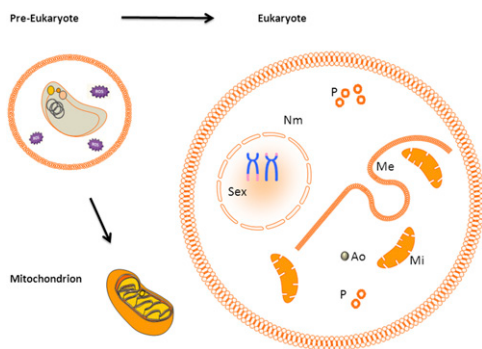


Fig. 3. Possible symbiogenic effects of internal ROS formation. Adaptations of the mitochondrion (Mi): supercomplex formation, antioxidant mechanisms, uncoupling (proteins), mitochondrial genome reduction, and carnitine shuttles. Adaptations of the cell: peroxisome formation (P), antioxidant mechanisms (Ao), internal membrane (Me) formation (autophagy), nuclear membrane (Nm) formation, size increase, and meiotic sex? See main text and ref. 103.

HAP2 is also missing from some familiar sexual taxa (notably Fungi and most Metazoa including humans; see Table S1 and ref. 53), where it has probably been replaced by newly evolved gamete fusion factors. Crustidiscoidea, Apusomonadida, and Ichthyosporidia are candidate asexual lineages (see above) that lack obvious homologs of both HAP2 and GEX1 in their sole genome-sequenced representatives (*Fonticula alba*, *Thecamonas trahens*, and *Sphaeroforma arctica*), but better sampling and more detailed analyses, including the application of the meiosis detection toolkit, are needed before we can claim their true asexual nature. From all of the prior observations we can safely conclude that meiotic sex in eukaryotes is the rule. It is very widespread, old, and clearly of major evolutionary importance.

Truly Asexual Eukaryotic (Micro)Organisms: Do They Exist for Long?

On the other hand, very few examples can be found where genome sequencing has led us to infer a strictly asexual nature of the respective eukaryote. One such case is *Adineta vaga*, whose genome sequence provided the final piece of evidence for the absence of meiosis and sex, probably in bdelloid rotifers (Metazoa) in general (56). The eustigmatophyte alga *Nannochloropsis oceanica* (Stramenopiles) was suggested to be ameiotic and asexual based on apparent monoploidy and relatively few meiosis-associated genes detected in its genome (57). However, analyses of the genome of a related species, *Nannochloropsis gaditana*, revealed a much more complete set of meiotic genes (58, 59), making *N. oceanica* only a candidate for relatively recent asexuality. *Emiliania huxleyi* (Haptophyta) is a globally distributed ecologically significant planktonic alga decorated by calcified scales. Occasionally, the dominant nonmotile diploid stage undergoes meiosis to create a motile differently scaled haploid stage (60). Remarkably, diploid-haploid switching is triggered by sensing specific *E. huxleyi* viruses (EhVs) that infect and destroy diploid, but not haploid cells (61). However, genomic evidence suggests that putative asexual lineages of *E. huxleyi* that lost the ability to switch to the haploid stage have recurrently emerged in populations inhabiting oligotrophic open oceans, perhaps as a consequence of low levels of EhVs in these environments (62).

There are more circumstances that would lead to protists foregoing sex. *Trypanosoma brucei evansi* is a subspecies of the African human-parasitic trypanosome that lost its extensive mitochondrial DNA (63). Compensatory mutations in the nuclear genome allow it to keep on clonally propagating in glucose-rich vertebrate blood (64), but as mitochondrial respiration is essential in its insect vector, it cannot be transmitted by a tsetse fly anymore, and is passed to another vertebrate via host's copulation. The well-documented meiotic event occurs only in the insect (11), making the subspecies asexual. Extensive analysis of a

set of *T. b. evansi* strains showed their multiple emergences from *T. brucei*, in time frames not even sufficiently long to precipitate the loss of meiotic genes (65). In the same vein, many truly asexual metazoans and plants have been described, but most of them seem to be recently derived lineages, closely related to sexual species, as well. Lineages believed to have lasted for millions of years are denoted "ancient asexuals." However, which of them, together with bdelloid rotifers, would qualify is highly contentious. For example, one of the candidate ancient asexual lineages was Glomeromycota (arbuscular mycorrhizal fungi) (66, 67). However, as mentioned above, genomic evidence for sex in at least some Glomeromycota species has been found recently (47, 48). Other candidates might be found among ciliates. This group is characterized by a unique cellular trait: the presence of an elaborate and intricate genomic architecture with morphologically and functionally differentiated germ-line (micronucleus) and somatic (macronucleus) nuclei. However, ciliates lacking a micronucleus, so-called amiconucleates, have been found, representing aberrant lineages within otherwise micronucleate species or even separate nominal species for which no micronucleate members are known. Given the fact that micronuclei are essential for ciliate sex and their loss seems irreversible, the amiconucleates most likely represent purely clonal lineages, some claimed to be millions of years old (68).

In summary, long-term asexuality is rare, and there is growing consensus that meiotic sex was present in the LECA and remains so in all major eukaryotic lineages (Fig. 2). If we accept the view that any costly nonadvantageous mechanism is subject to eventual loss, sex retention proves its usefulness, and bdelloid rotifers and ciliates, possibly having done without sex over an extended time period, might enlighten us regarding that use.

On the Advantages of Meiotic Sex

The benefits of eukaryotic meiotic sex are mostly discussed under the headings good, bad, and ugly: good, it generates genetic diversity in which rapid new combinations bring together beneficial mutations; bad, it purges the slow accumulation of slightly harmful mutations, i.e., it counters Muller's ratchet (69); ugly, it is necessary for the arms race with rapidly changing parasites, the well-known Red Queen hypothesis (70). In general, sex seems to allow superior responses to rapid challenges, both internal and external. Indeed, there is no consensus on a single major advantage for sex, and prevailing opinion is shifting toward a combination of advantages (71). That sex creates genetic variation, repairs DNA breaks, restores methylation patterns, and prevents accumulation of disadvantageous mutations is well accepted (72, 73). These features have varying importance in different eukaryotic lineages, yet it is likely that the advantages of sex, elaborated in detail for multicellular organisms (71, 74), either apply also for unicells or that there are some other advantages.

Although meiotic sex was lost in a few multicellular lineages millions of years ago (30) (see above), they constitute rare exceptions. These lineages represent easily amenable multicellular systems, and population genetic analysis showed that they do not suffer from the lack of genetic diversity, which is retained among different clones (75). It has been suggested that these asexual multicellular organisms may be better able to colonize diverse environments (76), and this presumption could be applied to (parasitic) protists as well. It has also been pointed out that for a single cell the costs of meiosis are high due to the time and effort it has to invest (77) and that, in protists, sexual reproduction is often associated with their survival of harsh periods (78). Under more constant optimal conditions, relatively simple organisms with short generation times are selected for high numbers of progeny, which can most efficiently be achieved by asexual reproduction (71). Thus, clonal reproduction may last for an extended period. However, when adverse conditions or internal constraints are encountered, sex is induced. This happens, e.g., in diatoms, where the construction of siliceous cell walls (frustules) makes them smaller with each mitotic division. This induces meiosis, gamete fusions, and restoration of the original cell size

by an expansion of the zygote (3). Some clonal populations will have salient characteristics. As aneuploidy and other chromosome aberrations may cause errors during meiosis, leading to dramatic decrease of fitness of progeny (79), one would not normally expect them to be retained. However, recent data obtained from parasitic flagellates such as *Leishmania* species (80) indicate that this may not apply for (some) temporarily clonal protists. Indeed mosaic aneuploidy, generally deleterious, is surprisingly well tolerated by *Leishmania* (80). Such oddities reflect unique adaptations produced by separate evolutionary histories and can be found in many protist lineages. The oddities observed, however, seem unlikely to be long lived. This is exemplified by *T. b. evansi* surviving loss of mitochondrial (kinetoplast) DNA by skipping the tsetse fly vector, in which it is needed, altogether (compare its loss of sex; see above). Based on almost zero mutation rates of hundreds of unused genes encoding mitochondrial proteins (65) with proven lack of essentiality (81), it has been proposed that after relatively short periods of time, these lineages vanish, being replaced by new strains that, via disruption of kinetoplast DNA (and hence of sex), entered the path to their slow, yet inevitable demise (65, 82).

Thus, despite the presence of organisms with long periods of clonality and occasional instances of aneuploidy and parasexuality, which deviate from regular standard meiotic sex, its option is still overwhelmingly present in practically all eukaryotes. Are the few examples of “real” longstanding clonality found in bdelloid rotifers and ciliates in agreement with the hypotheses for the maintenance of meiotic sex in general? We think the answer is yes. The ciliates can be considered as a maxiploidy system that uses the many gene copies of its macronucleus in a form of auto-recombination to combat accumulation of mutations and generate diversity (83, 84). However, one might wonder whether the amiconucleate population will in the end be as long lived as the one that still uses sexuality on encountering environmental stress. The bdelloid rotifers have more to tell us. Genomic analysis shows gene conversion, limiting the accumulation of mutations in absence of meiosis. Genes defending against transposons are increased, correlating with the fact that transposable elements make up only 3% of the genome. Horizontal gene transfer is very abundant, with 8% of the genes most likely acquired in this fashion. In this respect, bdelloids seem to have “returned” to the prokaryotic form of sex. A last salient observation: gene families involved in resistance to oxidative stress have been strongly expanded as well (see below) (56). All of this is in perfect agreement with theories regarding the benefits of sex as described above.

Did Meiotic Sex Evolve in Response to Reactive Oxygen Species?

To get full meiotic sex, a cell needs the following two basic mechanisms: (i) altering ploidy via cell-cell fusion and meiosis and (ii) mating type regulation of cell-cell fusion (in the beginning isogamous, later anisogamous). Further mechanisms involved (iii) coupling mating type regulation to (adaptive) diploid spore formation and (iv) coupling mating type regulation to (differential) organelle transmission. Many of the mechanisms involved can be envisaged to evolve rather easily from available prokaryotic mechanisms (85). To give but a few examples: meiosis-like processes evolved from homologous DNA repair, e.g., induced by reactive oxygen species (ROS). In this context, Spo11, an archaeal topoisomerase VI homolog that has lost its ligase ability and that introduces the essential double-stranded breaks, should be mentioned. Recombination at meiosis evolved, perhaps, as a repair mechanism of DNA strand damages (71). Gene exchange mediated by cell fusion seems to occur in some Archaea, at least in some instances triggered by stress conditions (86, 87). Finally, archaeal/bacterial changes in receptor molecule expression induced by stress conditions could, for example, evolve into mating type expression (85).

Lately, more and more research has uncovered links between stress conditions, such as ROS formation, and meiotic sex. Nedelcu showed that a large part of sexual inducer (SI) and several SI-induced extracellular matrix protein genes in

Volvox carteri are induced under various stress conditions (88). The same multicellular green alga cannot initiate its sexual cycle anymore in the presence of antioxidants. Furthermore, an iron chelator (iron being a cofactor in the formation of ROS) inhibits sexual induction in this species (89). Did sex evolve (in part) as a response to the DNA-damaging effects of ROS? Indeed, in 2013, Hörandl and Hadacek published “The oxidative damage initiation hypothesis for meiosis” (90). This also seems in agreement with the observations regarding bdelloids. To survive desiccation in cyclically drying freshwater habitats, they had to evolve extraordinary resistance to ionizing radiation and concomitant ROS, thus preventing extensive oxidative DNA damage and possibly rendering the meiotic sex repair mechanism superfluous in the process (56). This could imply that eukaryotes living in anoxic sediments or animal intestines (although these are challenged by other kinds of stress) might more easily become asexual. One essential question of course remains: where did the ROS that possibly gave rise to the invention of meiotic sex in eukaryotes come from?

Could Eukaryotic Sex be a Further Symbiogenic Effect due to the (Proto)Mitochondrion?

Recently, in a piece covering the “Symbiosis becoming permanent” meeting, Penissi discussed an assortment of highly interesting examples of intermediate stages between endosymbionts and organelles, which are purported to offer lessons for how the process played out early in life’s history (91). However, it is far from clear that this is in fact true for the first, and most momentous, endosymbiont to organelle conversion, that of an α -proteobacterium to the mitochondrion. The fact that all extant eukaryotes are derived from one kind of endosymbiotic organism that arrived relatively late on the evolutionary scene should make us reconsider the claim that “organelles are not so special,” when talking about mitochondria.

Eukaryotes only arose ~1.6–2.1 billion years ago (92), tellingly after the Great Oxygenation Event 2.3 billion years ago (93), compared with the “rapid” evolution of prokaryotes, which are more than 3.5 billion years old (94, 95). There are two contending models for the origin of mitochondria: the archezoan and the symbiogenesis models (96). The archezoan model states that an amitochondriate eukaryote took up the premitochondrion (in form of an alphaproteobacterium). Symbiogenesis states that the conversion of archaeon to eukaryote was the direct result of this uptake. The latest analyses fully support the eukaryote as a recent partnership between an archaeon and a bacterium (97). All later (secondary) organelle formations on further phagocytoses, such as chloroplast evolution and the many ongoing examples (91), would of course be relatively small feats in the second scenario and would not be informative about what happened during the primordial bacterium to mitochondrion conversion. Actually, the gradualist archezoan vision that seems to permeate recent thinking (91, 98) is much less likely than symbiogenic models, in our view.

A real problem for all models claiming primarily amitochondriate eukaryotes is their complete absence from present day life. A highly rapid, explosive period of adaptations as envisioned in symbiogenic models would explain their absence. Another difficulty, the large part of the mitochondrial proteome encoded by genes not directly recognized as being either derived from host or endosymbiont, seeming to be of unrelated bacterial or eukaryotic origin (98, 99), is also more easily understood as a product of intense, rapid adjustments to strong new evolutionary pressures on both genomes, leading to a plethora of novelties. Examples of such pressures are the possible influx of introns coming from the α -proteobacterial endosymbiont, leading to formation of the nucleus (100) or enhanced intracellular ROS formation leading among others to the evolution of peroxisomes (101, 102), intracellular compartmentalization, mitochondrial genome reduction and uncoupling, DNA protection by nuclear membrane formation,

Box 1. Eukaryote phylogenetic diversity: Reshuffling supergroups, microkingdoms, and an elusive root

Understanding the evolution of sex, as of any trait, requires getting to know phylogenetic relationships among taxa considered. We thus add a brief discussion on the main outlines of the eukaryote phylogeny portrayed in Fig. 2. We focus only on the most salient aspects and latest updates; interested readers may consult excellent recent reviews (112). A decade-old scheme based on molecular phylogenetic evidence, cytological features, and limited comparative genomic data divides nearly all extant eukaryotes into six major presumably monophyletic assemblages, so-called supergroups: Opisthokonta, Amoebozoa, Excavata, Archaeplastida, Chromalveolata, and Rhizaria (113). It was suggested that the root of the eukaryote phylogeny lies between Opisthokonta plus Amoebozoa on one side and the remaining supergroups on the other, with the clades on either side denoted unikonts and bikonts (114, 115). This scheme was adopted by the scientific community and taken for granted, even though improvements in phylogenetic methods and new genome-scale data from an ever-growing number of phylogenetically diverse lineages led to substantial modifications (2, 112). First, Chromalveolata is nonmonophyletic and has been superseded by a robustly supported grouping dubbed SAR, which integrates Rhizaria (demoted from supergroup status), but leaves out some chromalveolate lineages (haptophytes and cryptophytes) (2, 116, 117). SAR includes more protist species than all other eukaryotic groups combined and is extremely diverse in terms of cytology, body organization, and lifestyles. Haptophytes and cryptophytes plus several minor eukaryotic lineages were proposed to constitute a novel supergroup denoted Hacrobia or CCTH (118). However, monophyly of this grouping has not been supported in the latest analyses (117, 119), so it seems more appropriate to acknowledge at least five lineages with unresolved affinities: Cryptista, Haptophyta, Telonemia, Centrohelida, and Picozoa (previously called picobiliphytes) (120). Two lineages may need to be added: Microhelida, suggested to be related to Centrohelida, but with molecular phylogenetic evidence being inconclusive (121), and rappemonads, identified as a possible new deep lineage of algae in environmental surveys of plastid gene sequences, but possibly just representing a new class of haptophytes (122). Some of these lineages may be related to the SAR clade, whereas others may be related to, or even interrupt, the monophyly of the supergroup Archaeplastida (117, 119, 123). Second, a growing number of minor, but evolutionarily significant, lineages of flagellates, amoebae, or amoeboflagellates, referred to as microkingdoms, cannot be readily fitted into any of the supergroups (124). In addition to those mentioned (the different Hacrobia), the microkingdoms include Apusomonadida and Breviatea (recently united with Opisthokonta as a new major eukaryotic grouping dubbed Obazoa) (125), Ancyromonadida (=Planomonadida), Mantamonadida, Rigifilida, and Colodictyonida (=Diphyllatea). The latter four groups appear to be deeply diverged lineages branching close to Obazoa and the supergroup Amoebozoa (126–129), but their exact position is not known. Other such minor deep groups will surely be described, as their existence is suggested by culturing-independent environmental surveys of small subunit rRNA genes, such as the NAMAKO-1 and -2 lineages found in anoxic habitats (130). However, the majority of eukaryotic lineages of highest taxonomic rank seem to have been identified. Third, the original evidence for monophyly of unikonts and bikonts (and thus the eukaryotic root position) has been invalidated (131), and alternative rooting hypotheses have been suggested (132–136). The currently most convincing hypothesis with a well-defined methodology seems to be that of Derelle and Lang (137), recently corroborated by analyses of a larger and taxonomically better-sampled dataset (138). This hypothesis suggests the existence of two principal eukaryotic clades, called Opimoda and Diphoda, and implies that the traditional eukaryotic supergroup Excavata is paraphyletic or polyphyletic, as different lineages of Excavata are grouped with both Opimoda (Malawimonadida) and Diphoda (Discoba) (Fig. 2). The position of Metamonada, the third major lineage of Excavata, with respect to the root advocated by Derelle et al. (138), remains to be established. An important aspect of the recent rooting hypotheses is that all predict that the LECA was a complex, fully fledged eukaryote (96, 138, 139). The Opimoda-Diphoda rooting further suggests that the LECA was a biflagellated bacteriovorous excavate-like organism (resembling malawimonads and jakobids?) with a complex flagellum-associated cytoskeleton (138, 140). Significantly for this paper, sex, including meiosis, gamete fusion mediated by HAP2, and karyogamy mediated by GEX1, can be traced to the LECA under all recently proposed rooting hypotheses, even though evidence for the presence of sex and HAP2 and GEX1 orthologs is presently unavailable for many eukaryotic microkingdoms (Figs. 1 and 2).

and extensive increases in antioxidant mechanisms (103). Mounting evidence strongly indicates that peroxisomes are indeed a eukaryotic invention in response to entry of the premitochondrion (104–106). The symbiogenic model is also not plagued by a possible conceptual bioenergetics problem: how to pay for all of the expensive eukaryotic inventions without the presumably more efficient energy generation resulting from integrating different metabolic pathways and obtaining internal mitochondrial membranes (107). Again, coevolution seems to provide the answer. Maybe we have to recognize eukaryotic evolution as the product of α -proteobacterial endosymbiosis and the momentous, unlikely one-off, it possibly was.

Further Considerations and Conclusions

We speculate that apart from the adaptations mentioned above, eukaryotic meiotic sex also developed in the context of the sudden arrival of an endogenous ROS-generating entity that started making even more ROS because of its co-option for the efficient oxidation of different, alternating substrates. Indeed, general links between oxidative damage and the evolution of eukaryotic meiotic sex have been proposed before (86, 108–110). However, we add a new mechanistic aspect in the form of the kinetic model of ROS formation, which specifically states that shifts toward catabolic

substrates that use relatively more FADH_2 as electron donor during oxidation by the respiratory chain (substrates with a high $\text{FADH}_2/\text{NADH-F-N-}$ ratio, such as fatty acids) will increase internal ROS formation via reverse electron transport to complex I (101). This model explains the recent observation that ischemic reperfusion injury in organs is due to succinate accumulation on oxygen deprivation. After reperfusion, accumulated succinate (a high F/N substrate) is rapidly oxidized by succinate dehydrogenase, indeed driving extensive ROS generation by electrons flowing back to complex I (111). Could endogenous ROS formation by the endosymbiont be (one of) the ultimate cause(s) for the development of meiotic sex? Fig. 3 summarizes which eukaryotic inventions might have been influenced by endogenous ROS formation. Whether reactive oxygen has really been such a creative force remains to be seen.

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